COORDINATION OF IRON COPRO- AND ETIOPORPHYRINS WITH NITROGENOUS BASES

A dissertation submitted to the Board of University Studies, The Johns Hopkins University, in conformity with the requirement for the degree of Doctor of Philosophy

CARL SWENSSON VESTLING

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Foreword

Part I, which has to do chiefly with theoretical relations, is the joint contribution of Mr. Taylor, Mr. Davies, Mr. Vestling, and myself. Under this joint authorship it was published with the title *Metalloporphyrins. I. Coordination with nitrogenous bases. Theoretical relations* in The Journal of Biological Chemistry, **135,** 543 (1940).

Part II describes experimental results that were obtained by Mr. Vestling exclusively. It was published under his authorship with the title *Metalloporphyrins. IV. Coordination of iron copro- and etioporphyrins with nitrogenous bases* in The Journal of Biological Chemistry, **135,** 623 (1940).

W. Mansfield Clark

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PART I. THEORETICAL

In this series of studies we shall contribute to the systematic description of the behavior of metalloporphyrins in their coordination with nitrogenous bases.

The first method of attack will be the potentiometric. The results will be supplemented by spectrophotometric measurements.

Conant and his students (1, 2) initiated the potentiometric exploration of systems containing heme and a base that coordinates with heme in both the oxidized and reduced states. Such studies have been extended by Barron and Hastings (3) and particularly by Barron (4). Also important contributions to our knowledge of these complex systems have come by way of spectrophotometry, notably at the hands of Hogness, Zscheile, Sidwell, and Barron (5). The assembly of facts thus made available clarifies relations discussed in the earlier, enlightening papers of Anson and Mirsky (6) and Mirsky and Anson (7).

We shall deal with several systems containing not only different bases but metalloporphyrins composed of different metals and different porphyrins; therefore we require an adaptable nomenclature. We shall report the evidence of considerable complexity; therefore we think it wise to outline with care both the presumptions and the restrictions of a theoretical approach. We shall

have to submit our data to various sorts of analysis. Since that of any one sort will be repeated, we shall conserve space by devoting the greater part of this paper to the development of propositions that can be submitted to experimental tests and that may be referred to later by numbers. Exploratory measurements, detailed in the dissertations of Taylor (8), Davies (9), and Vestling (10) are summarized in the following papers by these authors.

Nomenclature

Several of the terms commonly employed in this field stem from those which arose during the early study of the prosthetic group of mammalian blood pigments and are not etymologically good when applied to substances containing metals other than iron and porphyrins other than protoporphyrin IX. Some attempts at reform have left the nomenclature in more or less confusion. We had adopted a simple nomenclature when Drabkin (11) proposed essentially the same system.

A compound formed from a porphyrin and a metal ion is called a metalloporphyrin. The metal and its state may be designated by expressions such as *ferro, cobalti,* etc., as in *manganomesoporphyrin IX.* A compound formed from a metalloporphyrin and another substance is designated by prefixing the name of the coordinating substance to that of the metalloporphyrin, as in *nicotine ferriprotoporphyrin.* In running commentaries, where the specific substance is clearly understood, a compound such as cyanide ferrohematoporphyrin may be spoken of as a base metalloporphyrin, *base* here being used in a wide sense.

Our symbols will refer to nitrogenous bases as coordinating substances but we do not wish to imply that only nitrogenous bases are able to coordinate with metalloporphyrins.

It will be assumed that the preparative procedures and certain identifying tests, which confirm what others have described, suffice to identify our porphyrins. If there be occasion to define a porphyrin quite specifically, both the name and the number given in Fischer's (12) system will be used, as in the name *mesoporphyrin IX.*

Scope and General Definition of Problems

Experience in this laboratory has confirmed our guess that some of the general features of these systems will be revealed by

modifying properties pertaining to one or another class of the components. Accordingly, porphyrin, metal, and coordinating base have been changed independently.

What was not foreseen is that when components are modified each of the systems so far studied has some property that restricts severely the range of experimental study. Accordingly the data require some piecing together if one is to obtain a general formulation of the equilibria and a tentative picture of the coordination compounds that are involved. In attempting this we find it essential to have in view the types of reaction that are to be met and equations formulating those under special study. Indeed, both the older data and much of that gathered in our first experiments are inadequate, because there were lacking those formulations that could have indicated critical relations. Also, such criticism of our final conclusions as may be made should involve a scrutiny of the basic assumptions.

The phenomena observed to date show that the following types of reaction occur in systems containing a metalloporphyrin and a coordinating base. The list is restricted to reactions that are pertinent to the immediate subject. As occasion demands, a more detailed formulation will be constructed with the accumulating evidence and a few postulates, and this will serve to account for the ionic charges not mentioned in the following primitive formulations.

1. Reversible association between porphyrin and reduced metal ion.

Example—Ferrous ion + protoporphyrin $\rightleftharpoons$ ferroprotoporphyrin

It will be recognized that this type of reaction, studied by Fischer, Treibs, and Zeile (13), is involved in the preparation of porphyrins from metalloporphyrins and of metalloporphyrins from porphyrins and metal ions. Except under the special conditions which Vestling (10) describes in his study of preparative methods, it will be assumed that the equilibrium state of this reaction is far "to the right." This assumption seems to be generally accepted for the alkaline water, and water-alcohol solutions such as were used in our studies and accordingly we have not examined it with the care that is desired ultimately.

That metal ions combine with porphyrins containing no acidic group other than $\rangle$NH is the most convincing of the evidences

that the predominating species of the metalloporphyrins having carboxyl groups are not normal salts of the carboxylic acids. Other evidence has confirmed the belief that they are coordination compounds involving the pyrryl nitrogens. This will be so far the tacit assumption that our formulations will provide for only one sort of metalloporphyrin, which we have already called a "coordination compound."

2. Reversible oxidation and reduction of the metalloporphyrin, if the metal component be of appropriate type.

Example– Cobaltimesoporphyrin + electron $\rightleftharpoons$ cobaltomesoporphyrin

It seems unnecessary to review the evidence that there is a preferential response to oxidizing and reducing agents, and that when these agents are suitably apportioned the first response is determined by the state of the metal component and that groups characteristic of the porphyrin alone are but slightly affected, if at all. Since each of the metals that we have used is such that 1 equivalent is concerned in the reduction of the quantity of metalloporphyrin containing a gm. atom of metal, formulations will be simplified by retaining this specification.

3. Reversible dissociation of hydrions (or hydroxyl ions).

Since porphyrin, metalloporphyrin, coordinating base, and base metalloporphyrin may have distinctive, or, in some cases, common groups with different acidic dissociation constants, there are numerous theoretical possibilities for the integration of the free energies of acid ionization and of hydrion dilution with the free energy of the ideal oxidation-reduction process. Actually, of course, all may be integrated in what may be called a continuum (Clark (14)). We may simplify matters greatly by anticipating the evidence that under the limited conditions of the measurements the detected, dissociable hydrions (or hydroxyl ions) pertain to the coordination center rather than to groups characteristic of the porphyrin alone or of the coordinating base alone. Ionizations pertaining to the latter two sorts of groups have to be considered under other circumstances. For the most part the range of pH used in the experimental studies was such that the carboxyl groups of the porphyrin can be *assumed* to be ionized.

4. Reversible association between oxidized metalloporphyrin and a nitrogenous base (including cyanide ion) and between reduced metalloporphyrin and the same base.

Examples—

Manganomesoporphyrin + r pyridine $\rightleftharpoons$ pyridine$_r$ manganomesoporphyrin

Manganimesoporphyrin + q pyridine $\rightleftharpoons$ pyridine$_q$ manganimesoporphyrin

It is the difference between the dissociation constants of the oxidized and reduced base metalloporphyrins that makes possible the potentiometric evaluation of these constants. This is the unique feature of the following papers the mathematical introduction of which is given in this paper.

5. Formation of polymers of metalloporphyrin or of a base metalloporphyrin.

On the one hand there will be given evidence of the existence of dimers in certain instances. On the other hand this calls for equations that are very complex and some of them are not amenable to the approximations that serve as guides to the experimental study of the simpler cases. None the less, when occasion demands, the simpler cases can serve as guides to the treatment of the complex.

For the study of a system having even a part of the possible complexity suggested by this compilation, spectrophotometry has one apparent, but not always real, advantage over potentiometry. The latter requires the presence of the oxidized and the reduced components, greatly increasing the number of components; yet electrode measurements provide certain basic data by means of which the free energy changes may be aligned with those of other oxidation-reduction systems. We need not foreshow the importance of this. What may be emphasized now is that each method has distinct limitations.

In order that the part contributed by each reaction to the free energy change of the continuum may be expressed in a manner that helps our understanding of these complex systems, we should have equations relating the experimentally determinable quantities.

A general equation would be unwieldy were it to describe the electrode potential as a function of the activities of all the components that are implied in this list of reactions. Therefore we had best break down the continuum of actually integrated processes and describe the parts by a plexus of equations. Happily, the control of hydrogen ion activities by means of buffer solutions permits us to eliminate consideration of the part played by hydrogen ion activity while developing other features and to deal

with the "pH effect" separately. Type equations for acid-base equilibria need no repetition.

Equations for Association

There remain for particular consideration the equilibria involving the associations between a nitrogenous base and a metalloporphyrin in the reduced and oxidized states. These are unique. Equations for a type case were first developed by Clark, Taylor, Davies, and Lewis (15) from the elementary equation of Conant and Tongberg (2). Only so much of the development will be given here as is necessary to outline the subject and to provide those typical equations that guide experiment. More detail will be found in the dissertations of Taylor (8), Davies (9), and Vestling (10).

Assume a metalloporphyrin that is subjected to oxidation-reduction in the sense

$$\mathbf{O}_n + ne \rightleftharpoons \mathrm{p}\mathbf{R}_m$$

where $\mathbf{O}_n$ represents the oxidant in the fixed state of aggregation designated by n, which is also the number of electrons, e, required for the reduction, and $\mathbf{R}_m$ represents the reductant in the fixed state of aggregation designated by m. If n be greater than 1, we shall now assume no intermediate state of reduction, although it will be important to reconsider this limitation should evidence make it necessary.

$$\mathrm{p}m = n \tag{1}$$

Let both species be under experimental conditions developing no fact that demands consideration of their acid ionizations or ionic charges, as would be necessary to "balance" the "equation" on one side of which appear n electrons. The electrode equation involving accepted conventions[1] is Equation 2.

$$E_h = E_0 + \frac{RT}{nF} \ln \frac{(\mathbf{O}_n)}{(\mathbf{R}_m)^{\mathrm{p}}} \tag{2}$$

Let a nitrogenous base, B, be added to the solution and there coordinate independently with the oxidized and the reduced

[1] See Clark (16).

metalloporphyrin to form the compounds defined by the following relations.

$$\mathrm{O}_n + q\mathrm{B} \rightleftharpoons \mathrm{O}_n\mathrm{B}_q$$

$$\mathrm{R}_m + r\mathrm{B} \rightleftharpoons \mathrm{R}_m\mathrm{B}_r$$

With brackets to indicate molar concentrations, parentheses to indicate activities, and γ to indicate activity coefficients we may state the equilibrium equations in two forms:

$$\frac{(\mathrm{O}_n)(\mathrm{B})^q}{(\mathrm{O}_n\mathrm{B}_q)} = K_O \qquad (3,\ a)$$

$$\frac{[\mathrm{O}_n][\mathrm{B}]^q}{[\mathrm{O}_n\mathrm{B}_q]} = \frac{\gamma_{O_n\mathrm{B}_q}}{\lambda_{O_n}\gamma_{\mathrm{B}}{}^q} K_O = K'_O \qquad (3,\ b)$$

$$\frac{(\mathrm{R}_m)(\mathrm{B})^r}{(\mathrm{R}_m\mathrm{B}_r)} = K_R \qquad (4,\ a)$$

$$\frac{[\mathrm{R}_m][\mathrm{B}]^r}{[\mathrm{R}_m\mathrm{B}_r]} = \frac{\gamma_{R_m\mathrm{B}_r}}{\gamma_{R_m}\gamma_{\mathrm{B}}{}^r} K_R = K'_R \qquad (4,\ b)$$

An important assumption is that there is no change in the state of aggregation when metalloporphyrin is converted to base metalloporphyrin.

The assumptions noted will deserve strict attention when the precision of measurement and control of all factors reach artistic adjustment to theoretical requirements. Some will receive further, qualitative treatment later.

Define the following sums

$$S_o = n[\mathrm{O}_n] + n[\mathrm{O}_n\mathrm{B}_q] \qquad (5)$$

$$S_r = m[\mathrm{R}_m] + m[\mathrm{R}_m\mathrm{B}_r] \qquad (6)$$

With the equations at hand derive Equation 7, *a*.

$$E_h = E_0 + \frac{RT}{nF}\ln\frac{S_o}{S_r{}^{\mathrm{p}}} + \frac{RT}{nF}\ln\frac{m^{\mathrm{p}}}{n} + \frac{RT}{nF}\ln\frac{K'_O}{(K'_R)^{\mathrm{p}}}$$

$$+ \frac{RT}{nF}\ln\frac{(K'_R + [\mathrm{B}]^r)^{\mathrm{p}}}{K'_O + [\mathrm{B}]^q} + \frac{RT}{nF}\ln\frac{\gamma_{O_n}}{\gamma_{R_m}{}^{\mathrm{p}}} \qquad (7,\ a)$$

If the conditions are such as to permit no serious change in the values of the activity coefficients occurring in Equations 3, *b*

and 4, *b*, we may proceed as if the last term in Equation 7, *a* were constant and K'_O and K'_R were constant. To simplify the typography we shall omit primes of K'_O and K'_R hereafter.

Proposition I—When all conditions are constant except the ratio of S_o to $(S_r)^p$, symmetry of the titration curve is presumptive evidence that $p = 1$. See also Proposition XII, *b*.

Proposition II—If $p = 1$, the slope of the titration curve gives the value of n.

Practically this is best determined by Proposition XVI. If $p = 1$, $m = n$. If it be found that $p = m = n = 1$ when sufficient base is present to convert the oxidized and reduced metalloporphyrins practically completely to the respective base metalloporphyrins, it will not necessarily hold that $p = m = n = 1$ for the metalloporphyrin system itself.

We shall continue with the assumption that $p = n = m = 1$ and equal activity coefficients. These assumptions reduce Equation 7, *a* to 7, *b*.

$$E_h = E_0 + 0.0601 \log \frac{S_o}{S_r} + 0.0601 \log \frac{K_O}{K_R} + 0.0601 \log \frac{K_R + [B]^r}{K_O + [B]^q} \quad (7, b)$$

There is included the condition that the temperature is 30°, since the consequences of the numerical coefficient are to be shown graphically.

If [B] be held practically constant, we have Equation 8, to be

$$E_h = E_b + 0.0601 \log \frac{S_o}{S_r} \quad (8)$$

tested by Propositions I and II. Here E_b indicates the potential that would be observed at 50 per cent reduction of the system at constant concentration of free base, which in most instances will obtain practically at constant concentration of total base. If no base be present, $E_b = E'_0$.

Proposition III—If the potential increase with addition of coordinating substance, all other conditions remaining constant, $K_O > K_R$. If the potential change is in the opposite direction, $K_R > K_O$.

We shall confine illustration to the first case.

Proposition IV—If on addition of base, all other conditions remaining constant, the potential approaches a limiting potential, E_2 (see Equation 11), it follows that $q = r$.

The difference $E_2 - E_0$ is determined by the ratio, K_O/K_R, as shown by Equation 11 and as illustrated in Fig. 1. The course of the association curve between E_0 and E_2 is determined by the absolute values of K_O and K_R, and by the concentration of total metalloporphyrin, S.

Particular emphasis may be given to one conclusion that flows from a consideration of these systems and that is implicit in the equations. This is that the change of potential, accompanying the addition of a coordinating base to a fixed mixture of the reduced and oxidized species of the metalloporphyrin, is a measure of the *ratio* of the two dissociation constants. This difference betokens a difference in the free energies of association and wheres this is large it may need to be considered in the ultimate analysi of the part that complexes of the metalloporphyrins play in catalysis. Furthermore, the fact that other catalysts found in living cells depend upon the association between dissociable prosthetic groups and specific proteins suggests that base metalloporphyrin systems may serve in some degree as models of important general relations. Barron (4) has already pointed to the fact that the potentials of systems containing the same metalloporphyrin can vary considerably with the nature of the base. These specific effects of coordinating bases are referable to the specific values of K_O and K_R. Barron and Hastings (3) and Barron, DeMeio, and Klemperer (17) have applied the shift of potential to the modification of catalytic action.

It is desired to develop Equation 7, b to include the concentration of total coordinating base, S_b, which is directly determinable. The concentration of total base is defined by Equation 9. Other definitions and specifications convenient to the development follow.

$$S_b = [\mathrm{B}] + q[\mathrm{OB}_q] + r[\mathrm{RB}_r] \tag{9}$$

$$S = S_o + S_r \tag{10}$$

$$E_2 = E_0 + 0.0601 \log \frac{K_O}{K_R} \tag{11}$$

$$x = \frac{[O]}{[R]} = \log^{-1} \frac{(E_h - E_0)}{0.0601} \tag{12}$$

$$y = \frac{[OB_q]}{[RB_r]} = \log^{-1} \frac{(E_h - E_2)}{0.0601} \tag{13}$$

$$\alpha = \frac{S_o}{S} \tag{14}$$

$$\beta = \frac{[OB_q] + [RB_r]}{S} \tag{15}$$

With these relations Equation 16 may be derived.

$$E_h = E_0 + 0.0601 \log \frac{S_o}{S_r} + 0.0601 \log \frac{K_O}{K_R} + 0.0601 \log \frac{K_R + (S_b - ZS)^r}{K_O + (S_b - ZS)^q} \tag{16}$$

where, for the restriction that $q = r$

$$Z = \frac{r(y+1)}{(x-y)}[x(1-\alpha) - \alpha] \tag{17}$$

and for the further restrictions that $\alpha = 0.5$ and $r = 2$

$$Z = \frac{(y+1)(x-1)}{(x-y)} \tag{18}$$

For the restriction that $\alpha = 0.5$ but no limitation on the relation of q to r

$$Z = \frac{(qy+r)(x-1)}{(x-y)} \tag{19}$$

Proceed, for the moment, with Equations 16 and 18.

Proposition V—Certain first order, graphical approximations may give useful information, as detailed below.

In the case selected, unique points of the association curve are (*a*) where

$$[B]^2 = K_R \text{ or } 2 \log [B] = \log K_R \tag{20}$$

There $Z = 0.5$, $E_h - E_0 \fallingdotseq 0.0181$, $S_b \fallingdotseq K_R - 0.5S$. The last two relations and the known values of S_b and S make it possible to judge when $S_b \fallingdotseq [B]$. This approximation is least valid at the low parts of the association curve, but, especially when the value of S is low, it will be found frequently that it may be used profit-

ably if not extended below this first unique point. Then S_b may replace [B] in Equation 7, *b* and modified Equation 7, *b* may be used in place of the unwieldy Equation 16; (*b*) the center point of inflection where

$$[B]^4 = K_O K_R \tag{21}$$

There $Z = 1$, $[B] = (S_b - S)$, $\beta = 0.5$, $[RB_r] = [O]$, $[R] = [OB_q]$, and $E_h = (E_0 + E_2)/2$; (*c*) where

$$[B]^2 = K_O \text{ or } 2 \log [B] = \log K_O \tag{22}$$

There $Z = 1.5$ and $E_2 - E_h = 0.0181$.

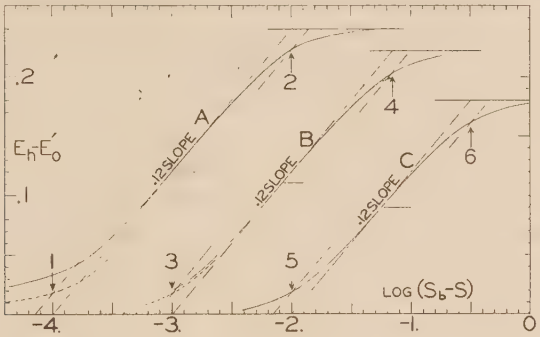

FIG. 1. The solid lines represent the theoretical relation of $E_h - E'_0$ to log $(S_b - S)$ when $q = r = 2$, $0.5S = S_r = S_o = 1 \times 10^{-4}$ M; the broken lines, the same with log $(S_b - 0.5S)$ as abscissa. Curve A, $K_R = 1 \times 10^{-8}$, 0.5 log $K_R = -4.00$ (see Point 1); $K_O = 1 \times 10^{-4}$, 0.5 log $K_O = -2.00$ (see Point 2); $E_2 - E_0 = 0.2400$ (0.06 coefficient). Curve B, $K_R = 1 \times 10^{-6}$, 0.5 log $K_R = -3.00$ (see Point 3); $K_O = 5 \times 10^{-3}$, 0.5 log $K_O = -1.15$ (see Point 4); $E_2 - E_0 = 0.2219$ (0.06 coefficient). Curve C, $K_R = 1 \times 10^{-4}$, 0.5 log $K_R = -2.00$ (see Point 5); $K_O = 1 \times 10^{-1}$, 0.5 log $K_O = -0.50$ (see Point 6); $E_2 - E_0 = 0.1800$ (0.06 coefficient).

For the case under consideration it may be shown that, when $K_O > S_b{}^r > K_R$ at the center point, the slope at the mid-point approaches the uniquely simple, algebraic form (Equation 23):

$$\frac{dE}{d \log(S_b - S)} \doteq (0.0601)r \tag{23}$$

Fig. 1 illustrates a method of graphical analysis based upon the use of these approximations and unique points. Since Z is

unity at the center point, log $(S_b - S)$ is made the abscissa in order that the important orienting diagonal, of slope 0.12, may coincide with the center point. When the ratio of K_O to K_R is as large as any of the values indicated, the diagonal is very close to the tangent through the mid-point and accordingly can be well placed. This diagonal will intersect the line of E'_0 where

$$0.5 \log K_R = \log [B] \tag{24}$$

Now if $[B] \fallingdotseq (S_b - S)$, as it does in Curve C, this point of intersection will be 0.0181 volt below the corresponding point of the association curve, drawn with log $(S_b - S)$ as abscissa. Curves B and A show progressively greater departure from this relation. Yet at the unique point in question $Z = 0.5$, so that, if a supplementary curve be plotted with log $(S_b - 0.5S)$ as abscissa, the unique point falls on the supplementary curve very close to 0.0181 volt above the aforementioned intersection. We shall show that it is not always possible to report a reliable value for E'_0. When deprived of this base-line, one may use the obvious, alternative method shown in Fig. 1; namely, to intersect the appropriate curve with a line parallel to the orienting diagonal and 0.0181 volt above. For the curves shown and on the scale used in Fig. 1 it would be difficult to discern any difference along the upper part of the curves whether the abscissa were made log $(S_b - S)$, log S_b, or the log $(S_b - 1.5S)$ which is demanded for the location of the unique point where

$$0.5 \log K_O = \log [B] \tag{25}$$

If necessary a drawing of larger scale may be used and the principle described for the finding of K_R applied to the estimation of K_O. Fig. 1 illustrates the elements.

Once the orders of magnitude of K_O and K_R, as well as of S, are known, one may estimate the legitimacy of each approximation. In extreme cases it may be necessary to resort to a series of progressively closer approximations but, especially when S is small and the constants large, it suffices to plot with log S_b. The latter is preferable for final description.

It should not go unnoted that previous authors have plotted with S_b as abscissa. The resulting graph obscures those features that are useful in the estimation of K_R and K_O and it may leave

the impression that the potentials at high concentration of base have reached a limit when the evidence thereof is quite inadequate.

Proposition VI—If at and near the center of an association curve K_O is much greater than $[B]^q$ and K_R is much less than $[B]^r$, the value of r will be determined by the maximal slope of the association curve, which is given by Equation 23.

The actual slope need only approximate an integral multiple of 0.0601, since r must be a whole number. The departure will be the greater the smaller the ratio of K_O to K_R. If q be less than r, the effect of combination between base and oxidant will extend over a larger range and may affect the slope of the midsection.

Comment on Stepwise Association—If association be stepwise, a term such as $K_O + [B]^2$ in Equation 7, b must be replaced by

$$K_{O_1}K_{O_2} + K_{O_1}[B] + [B]^2 \qquad (26)$$

or by a more extended term if the number of steps be greater than two. On statistical grounds stepwise association is more probable than concurrent addition of 2 or more molecules of base and under certain distributions of the energy changes this may be reflected in the equilibrium states. Therefore the theorist will be tempted to provide for this. On the other hand the experimentalist will recognize that the provision of several constants in terms of this kind is not unlike the provision of several constants in those infinite series that are used for the development of empirical equations and becomes devoid of physical significance if his method be not delicate enough to detect the individual values. But be it noted that, as [B] varies, the value of the sum in Equation 26 runs from that of the first term to that of the last. Accordingly, for the purpose of defining the gross contour of an association curve, Equation 26 may be reduced to the corresponding term in Equation 7, b.

The further the departure between the values of the first and second dissociation constants, the more will be the weight of a $K_1[B]$ term, so that experimental identification may become possible. When it is, the equations may be enlarged appropriately.

A Note on Polymers—The potentiometric method can reveal

the presence of polymers. A particular case was described by Stiehler and Clark (18). Barron (4) and Davies (9) show that the components of certain metalloporphyrin systems are dimers. The equations here given are predicated on the assumption that there is no *change* in state of aggregation and where the symbols that have been used imply monomeric components the equations need only slight modification to represent the case in which all metalloporphyrins are dimeric. The situation is entirely different when there is a *change* in state of aggregation accompanying association with a base. Because of the much greater complexity

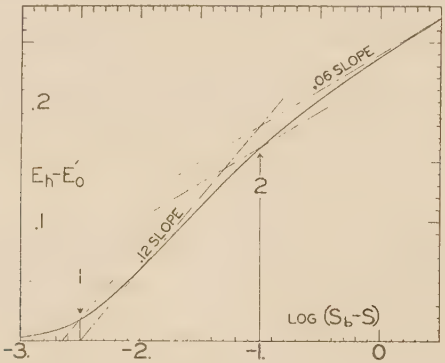

FIG. 2. Theoretical curve. Case of $q = 1$, $r = 2$, $K_R = 1 \times 10^{-5}$, $K_O = 1 \times 10^{-1}$. Point 1, 0.5 log K_R = log $(S_b - S)$; Point 2, log K_O = log $(S_b - S)$.

of such equations we think it wise to postpone their treatment until necessity demands it.

Cases in Which $q < r$ – For such cases Propositions I, II, III, and VI will hold, while IV and V need modification. Fig. 2 illustrates a theoretical case and a method of graphical analysis so similar to that already described as to require no further comment. Fig. 2 will also serve to illustrate some of the propositions that follow.

Proposition VII—If $q < r$, the maintenance of the slope r (0.0601) at high values of [B] should indicate that K_o is infinite, and $q = 0$ (no association of oxidized metalloporphyrin and base).

Proposition VIII—A decline of the slope on further addition of base indicates that K_o is finite and $q > 0$.

On still further addition of base the term 0.0601 log $(K_R + [B]^r)/(K_o + [B]^q)$ approaches 0.0601 log $[B]^r/[B]^q$ or 0.0601 log $(S_b)^{r-q}$ (see Fig. 2). Whence:

Proposition IX—If, at very high concentration of coordinating base, the slope of the association curve approaches a constant value, the slope is given by Equation 27 from which is determined the value of $r - q$.

$$\frac{\Delta E_h}{\Delta \log S_b} = 0.0601(r - q) \qquad (27)$$

Participation of Hydrions—To avoid unnecessary complexity we have followed the policy of breaking down the continuum of actually integrated processes and up to this point have left out of consideration the participation of hydrogen (or hydroxyl) ions. That this is a practical policy flows from the established fact that, if a set of measurements be made in buffer solutions at constant pH, the components of the buffer system will absorb or supply the hydrogen ions liberated or taken up in the reactions hitherto considered and no experimental evidence of their participation will appear, provided, of course, that the buffer system be in great excess. By the same token, a shift in the pH value of the buffer system will alter the ease with which hydrogen ions are made available for the completion of the reaction and the effect will appear in the magnitude of the over-all free energy change, the intensity factor of which is measured by the electrode method.

Since these principles have been developed in previous studies (19), it seems unnecessary to embody them in equations now being used to outline general features, although it will be essential to do so in dealing with specific, actual cases. But there is one aspect that should be given particular emphasis lest the policy now being followed become grievously deceptive.

Prior to potentiometric studies by Conant (1, 2) and Barron (4) it was largely a matter of artistry to assign to ferriprotoporphyrin in alkaline solution a hydroxyl ion, or a species formula that we shall represent by $O\overline{OH}$. Now that potentiometric measurements have shown hydrogen ions or hydroxyl ions to be involved in the equilibria obtaining among some of the systems, we must contemplate the possibility that there is a competition between hydroxyl ions and coordinating base molecules for posi-

tion at the coordination center. To take care of this in the simplest of several possible formulations let us postulate that in the coordination between base and oxidized metalloporphyrin the base coordinates only with the species **O**, leaving applicable Equation 3, *b* but that, in addition, there obtains the equilibrium represented in Equation 3, *c*.

$$\frac{[\mathbf{OOH}][H^+]}{[\mathbf{O}]} = k[H_2O] = K_h \qquad (3, c)$$

By following the usual procedure with the inclusion of Equation 3, *c* one arrives at an "electrode equation" comparable with Equation 7, *b* and differing therefrom only in the last term. This term when written in a form suited to the immediate discussion is

$$0.0601 \log \frac{K_R + [B]^r}{\frac{K_O}{[H^+]}([H^+] + K_h) + [B]^q} \qquad (28)$$

The point to be emphasized is that when association experiments are run at *constant* hydrogen ion concentration the constant called K_O in the foregoing discussion should be in reality $(K_O/[H^+])([H^+] + K_h)$. In other words what has been called the dissociation constant of the base metalloporphyrin in oxidized form is an "apparent constant" the value of which is a function of $[H^+]$.

The general significance of this warning is not dependent upon the particular postulate used above--a postulate which we shall show is too simple.

Proposition X—Previous theoretical and experimental studies (19, 20) have shown that, when the relation between E_h and pH, other conditions being constant, is

$$\frac{-\Delta E_h}{\Delta \text{ pH}} = 0.0601 \text{ (at } 30°) \qquad (29)$$

the reductant must possess 1 more hydrogen ion (or 1 less hydroxyl ion) than the oxidant for each electron necessary to convert oxidant to reductant.

Evidence will be presented in the experimental papers that the pH effects have to do with the coordination shell so that we may now say that the above relation will demonstrate the reduced

coordination center to possess 1 more hydrogen ion or 1 less hydroxyl ion per metal atom.

Proposition XI—On increase of pH, if the *reductant* lose a equivalents of protons per mole, the value of $-dE_h/d$ pH will decrease by $a(0.0601)/n$ (at 30°); while if the *oxidant* lose a equivalents of protons per mole the value of $-dE_h/d$ pH will increase by $a(0.0601)/n$. Loss of a proton is equivalent to gain of hydroxyl ion. See references (19) and (20) for details on this proposition.

An example will be shown of a change from $-\Delta E_h/\Delta$ pH = 0.0601, for the higher values of pH, to $-\Delta E_h/\Delta$ pH = 0.0 for the lower values of pH. Whatever the structural interpretation may be, this experimental fact demands the *equivalent* of the loss of hydroxyl ion from the oxidant at the lower pH values. Also the mid-point of the transition between the two slopes gives the so called pK value.

Comment on Activities—There remain for consideration troublesome questions arising from changes in activity coefficients that have been neglected up to this point. A rigid treatment of this subject, involving so complicated a set of components, would require a length out of proportion to the scope of the experiments to be reported. Therefore, we may reduce our considerations to the following brief summary of our policy regarding the least certain of the several experimental aspects of this subject.

Simultaneous control of all factors but the one under immediate investigation is not rigidly attained in actual experiments. The greater uncertainties lie in the interpretation of data purporting to yield "pH" values and in corrections for those changes of pH and other factors that attend the modification of the medium. To provide a basis for the discussion of the first it seems advisable to outline here the experimental procedure.

pH Control—In many cases the concentration of added base, such as pyridine or nicotine, is so large as to make impracticable the use of the pH numbers of the solutions containing only the buffer salts. Furthermore, such substances interfere with the proper operation of the hydrogen electrode in the solutions used. Accordingly the glass electrode was employed. The type introduced by MacInnes and Belcher (21) was operated in a shielded, constant temperature, air bath. A Compton quadrant electrome-

ter was used for null point instrument in conjunction with a Leeds and Northrup type K potentiometer. A very close approach to the theoretical change of potential with pH was established with acid and neutral buffers that had been measured with the hydrogen electrode. The glass membrane had to be used with alkaline solutions with which there are well known difficulties. The use of potassium in place of sodium in the buffers materially reduced drifts and deviations. Since the membrane of Corning No. 015 glass behaved normally with mildly acidic buffers, if previous contact with alkaline buffers had not been prolonged, it was assumed that its initial response in alkaline solution was the more reliable. Accordingly rapid measurements were made of the tested solution and of buffer solutions of nearly the same pH numbers, as determined by the hydrogen electrode, and interpolations were made. By this procedure there were found orderly relations between the potentials of the cell with glass membrane and the amounts of alkali added to any one of the various buffer solutions and also orderly relations between the potentials and the amounts of pyridine or alcohol added to aqueous buffers.

If we accept a potential so measured as indicative of a pH number, that number appears to be referred to the standard for aqueous solutions and as if measured with the hydrogen electrode, since the latter remained the means of standardizing the compared solutions. Actually the higher concentrations of pyridine, or nicotine, which in some cases went as high as 3 M, may seriously impair the ordinary interpretation of "pH" and of the standard methods of its computation. Nevertheless, if any one set of data be restricted to solutions having grossly the same composition and if it then be found, on making a "pH" change the major variable, that there is established a close approach to the pH effect predicted by the simplified equations, the following conclusion is drawn. While strict standardization of pH numbers is precluded, the experimental satisfaction of the predicted pH effect in terms of *relative* pH changes establishes the *nature* of a part of the equilibrium. This, rather than precisely referred values, we consider to be of first order importance.

The greater uncertainties are in the experiments purporting to show the course of association. Here occur the largest of the uncontrolled changes of the medium. What we have been forced

to assume are the following. (*a*) The shift of pH accompanying the addition of neutral pyridine, nicotine, etc., is given by glass electrode measurements and can be applied by use of the established pH effect so as to bring all points on the association curve to the basis of constant "pH" on the arbitrary scale. (*b*) The shift of the activity coefficients is *assumed* to be negligible while coordinating base is increased. Here we may retain the same order of magnitude of the ionic strength but the dielectric constant will change.

APPLICATIONS OF SPECTROPHOTOMETRY[2]

Granting that obvious prerequisites for spectrophotometry are met and that *only two* species containing metalloporphyrin absorb at a suitably chosen wave-length, we may use the following relations.

Assume the process

$$aM_m + bA \rightleftharpoons cM_nA_e \tag{30}$$

where A represents either (*a*) base coordinating with M_m at constant pH to form M_nA_e or (*b*) hydrion adding to M_m to form M_nA_e at constant concentration of base. In case (*b*), M may be either metalloporphyrin or base metalloporphyrin. Relation 30 is stated in this general form for economy of space and to avoid needless repetition for the several specific cases. The restriction to two absorbing species places a severe limitation upon the following relations. Relief from this restriction will be noted in a special, experimental case.

Implied in Relation 30 are that:

$$am = cn \tag{31}$$

$$b = ce \tag{32}$$

$$\frac{[M_m]^a[A]^b}{[M_nA_e]^c} = K \tag{33}$$

Define

$$S = m[M_m] + n[M_nA_e] \tag{34}$$

$$\epsilon' = -\log T \tag{35}$$

[2] See also Hogness *et al.* (5).

where T is the transmittance for any mixture of absorbing species at a fixed value of S and given length of absorbing column.[3]

$$\epsilon'_1 = -\log T_{Mm} \tag{36}$$

where T_{Mm} is the limiting value of T where $S = m[M_m]$.

$$\epsilon'_2 = -\log T_{MnAe} \tag{37}$$

where T_{MnAe} is the limiting value of T when $S = n[M_nA_e]$. By application of the rule of the additive property of log transmittances one obtains

$$\frac{[M_m]^a}{[M_nA_e]^c} = \frac{K}{[A]^b} = S^{a-c}n^cm^{-a}(\epsilon'_1 - \epsilon'_2)^{c-a}\frac{(\epsilon'_2 - \epsilon')^a}{(\epsilon' - \epsilon'_1)^c} \tag{38}$$

Proposition XII—(*a*) Symmetry of the curve relating ϵ' to log $[A]$ is presumptive evidence that $a = c$, whence $m = n$.

(*b*) Absence of a "dilution effect" involving S^{a-c} is further evidence. When $a = c$ and $m = n$, Equation 38 reduces to Equation 41 with appropriate interpretations of b and K.

If S is constant, the tangent to the curve at the mid-point, where $\epsilon' - \epsilon'_2 = \epsilon'_1 - \epsilon'$, will be given by

$$\frac{d\log[A]}{d\epsilon'} = \frac{-0.8686}{(\epsilon'_1 - \epsilon'_2)}\left(\frac{a+c}{b}\right) \tag{39}$$

Practically the use of this tangent may not be very certain, but to illustrate uses, consider the following possible cases.

Case 1. $M + 2A \rightleftharpoons MA_2$; $a = 1$, $b = 2$, $c = 1$
" 2. $2M + 3A \rightleftharpoons M_2A_3$; " = 2, " = 3, " = 1
" 3. $M_2 + 2A \rightleftharpoons M_2A_2$; " = 1, " = 2, " = 1

All of these give the same value of $(a + c)/b$. Yet Cases 2 and 3 would require 2 equivalents for the oxidation-reduction of the *base* metalloporphyrin. Therefore, if a symmetrical, potentiometric curve involving 1 equivalent were to be found, Case 1 could be presumed to apply and the slope now under discussion would indicate that $b = 2$. An additional and better test for

[3] The symbol ϵ' is introduced for economy of space in the more complicated equations. With apologies to the reader we ask that he carefully distinguish ϵ' from ϵ (see Table I).

the value of b is discussed in the following section. Be it noted that the spectrophotometric test alone would not distinguish Case 1 from Case 3.

Proposition XIII—If $a = c$ the center of the symmetrical curve relating ϵ' to log $[A]$ is where $\epsilon'_2 - \epsilon' = \epsilon' - \epsilon'_1$ and at this point

$$\log K = b \log [A] \tag{40}$$

RECTIFICATION OF CURVES

The general method of Reed and Berkson (22) is used.

Rectification of Spectrophotometric Curves. Relation of $-$ *Log* T (*or* ϵ') *to Log A* —This is limited to the following modification of Equation 38.

$$\frac{[M_m]}{[M_m A_b]} = \frac{K}{[A]^b} = \frac{\epsilon'_2 - \epsilon'}{\epsilon' - \epsilon'_1} \tag{41}$$

Choose an orienting pair of values called ϵ'_0 and log $[A]_0$ and define

$$b \log [A] - b \log [A]_0 = p \tag{42}$$

$$\log K - b \log [A]_0 = -\log G \tag{43}$$

where $p = 0$, when $\epsilon' = \epsilon'_0$. Derive

$$G\, 10^p = \frac{\epsilon' - \epsilon'_1}{\epsilon'_2 - \epsilon'} \tag{44}$$

$$G = \frac{\epsilon'_0 - \epsilon'_1}{\epsilon'_2 - \epsilon'_0} \tag{45}$$

Whence

$$\epsilon' = \epsilon'_1 + (G + 1) \frac{(\epsilon'_0 - \epsilon')10^p}{1 - 10^p} \tag{46}$$

Proposition XIV—If the assumed value of b fits the actual case, a chart of ϵ' against $((\epsilon'_0 - \epsilon')10^p)/(1 - 10^p)$ should give a straight line the properties of which determine K, ϵ'_1, and ϵ'_2.

The slope is $(G + 1)$, whence K is found by Equation 43. The intersection with the ϵ' axis gives ϵ'_1. Thence ϵ'_2 is found by Equation 45. If desired, the equation can be recast to yield ϵ'_2 by intersection.

Rectification of Potentiometric Association Curves Relating E_h to Log S_b--Let Equation 7, b be solved for $[B]^q$ under the condition that $q = r$. Assume $[B] \fallingdotseq S_b$. Define

$$\log^{-1} \frac{E_h}{0.0601} = u \tag{47}$$

$$\log^{-1} \frac{E_2}{0.0601} = J \tag{48}$$

$$q \log S_b - q \log S_{bo} = p \tag{49}$$

$$-q \log S_{bo} = \log C \tag{50}$$

The chosen pair of orienting data are u_o and S_{bo} where $p = 0$. Derive

$$u = J + (CK_O + 1) \frac{u_o - u}{10^p - 1} \tag{51}$$

Proposition XV—When previous knowledge justifies the use of the approximation carried into Equation 51, the slope of the straight line relating u to $(u_o - u)/(10^p - 1)$ is $(CK_O + 1)$ which yields the value of K_O by use of Equation 50 and the intersection with the u axis yields E_2 by Equation 48.

Similar relations leading to the evaluation of K_R and E'_0 could be obtained by use of $u' = \log^{-1} (-E_h/0.0601)$ and $J' = \log^{-1} (-E_0/0.0601)$.

The use of this method of rectification is tricky for the following reasons.

If the orienting values of u_o and $\log S_{bo}$ be taken from near the center of the association curve where the effects of K_O and K_R are small, the slope of the rectified line approaches unity and is of no value in the determination of either constant. The approximation carried into the equation, namely $S_b \fallingdotseq [B]$, is least valid at the lower part of the association curve. Therefore we hesitate to use this method for evaluating K_R. The following simpler rectification may be applicable in rare cases. Assume $q = r$, $S_o = S_r$, $S_b = [B]$, $K_O >> [B]^q$. Whence $E_h = E'_0 + 0.0601 \log (K_R + S_b^r)/K_R$. Define $u = \log^{-1} (E_h/0.0601)$ and $E_0 = 0.0601 \log W$. Whence $u = W + (W/K_R) S_b^r$. Thus a plot of u against S_b^r should approximate a straight line the proper-

ties of which allow estimate of K_R but only a crude estimate of E'_0.

Rectification of Titration Curves

Reed and Berkson's method as applied to this case has been discussed by Clark and Perkins (23). We shall use the symbols defined by the latter authors as follows:

$y \equiv$ number of ml. of specified reducing agent required to reach the potential E_h
$d \equiv$ number of ml. of the reducing agent added before the main system is attacked
$A \equiv$ number of ml. of the reducing agent required to carry the main component from 0 to 100 per cent reduction
E_n and y_n are chosen orienting values of E_h and y respectively

$$P = \frac{E_h + E_n}{1/n(0.0601)} \tag{52}$$

$$E'_0 + E_n = -\frac{0.0601}{n} \log C \tag{53}$$

Whence

$$y = (C + 1)\frac{y_n + y10^P}{1 - 10^P} - dC \tag{54}$$

Proposition XVI—The properties of a titration curve are determined by the properties of a straight line the coordinates of which are y and $(y_n + y10^P)/(1 - 10^P)$ according to Equation 54.

The mid-point potential, E'_0, is determined by the slope $(C + 1)$ and thence all other characteristics of the titration curve are evaluated as described by Clark and Perkins.

The method is predicated on the applicability of Equation 8 and in particular upon an integral value of n. It is important to note the following. It is possible that the method may yield such numerical values of the constants that a theoretical, sigmoid curve, constructed with these values, may be shifted to a position of best correspondence with an actual titration curve that is slightly asymmetric. To be sure, only a slight asymmetry would go undetected; nevertheless there remains danger of overlooking something. For the present we may say that we know

of no better criterion of the validity of deduced values to be reported unless it be so extreme a precision of measurement and so rigid a control of variables as to make the mathematical analysis by the method of Reed and Berkson superfluous. All titration data are analyzed by this method.

SUMMARY

A nomenclature for metalloporphyrins and their coordination compounds is proposed.

The reactions that may take place in systems containing metalloporphyrins and coordinating bases are typed to define the restrictions of the immediate study.

There are developed theoretical equations relating the electrode potential, the concentration of total metalloporphyrin, the total base that coordinates independently with reduced and oxidized metalloporphyrin, and the ratio of concentrations of oxidized and reduced metalloporphyrin.

A brief review is given of the principles governing the study of the part played by the acid-base equilibria and of experimental difficulties in the application of these principles to the cases at hand.

Approximate forms of the equations are developed to show graphical methods of determining the constants that are descriptive of the dissociation of oxidized and reduced coordination complexes and to show methods of determining the numbers of molecules of coordinating substance that associate on the one hand with reduced metalloporphyrin and on the other hand with oxidized metalloporphyrin.

Equations are given for the use of spectrophotometric data in determining the various constants that define the equilibria of the systems.

Equations are given for rectification of curves.

Sixteen propositions and corollaries thereto are offered as subject to the experimental tests applied in the subsequent papers.

TABLE I

List of More Important Symbols

[B] = molar concentration of free, uncombined, coordinating base
E_h = electrode potential referred to the hydrogen standard
E_b = E_h at constant S_b, other conditions being specified in the text
E'_0 = E_h at 50% reduction of metalloporphyrin, $S_b = 0$, other conditions being specified in the text
e = 1 equivalent of electrons
F = the faraday
K_O = dissociation constant of oxidized base metalloporphyrin
K_R = dissociation constant of reduced base metalloporphyrin. Note, special dissociation constants are defined as needed
n = (1) number of equivalents required to reduce 1 mole of oxidant; (2) number of gm. atoms of metal per mole of oxidant
$[\mathbf{O}_n]$ = molar concentration of oxidized metalloporphyrin containing n gm. atoms of metal ion per mole
[OOH] = molar concentration of oxidized metalloporphyrin associated with 1 mole of hydroxyl ions per mole
$[\mathrm{B}_q\mathbf{O}_n]$ = molar concentration of oxidized, base metalloporphyrin containing q moles of base per mole
$\epsilon' = -\log \mathrm{T} = \epsilon cl$
p = ratio of gm. atoms of metal per mole of oxidant to gm. atoms of metal per mole of reductant
q = number of moles of base associating with 1 mole of oxidized metalloporphyrin
r = number of moles of base associating with 1 mole of reduced metalloporphyrin
$[\mathbf{R}_m]$ = molar concentration of reduced metalloporphyrin
$[\mathrm{B}_r\mathbf{R}_m]$ = molar concentration of base metalloporphyrin containing r moles of base per mole
R = gas constant
$S = S_o + S_r$
S_o = sum of apparent molar concentrations of oxidized metalloporphyrin and oxidized base metalloporphyrins
S_r = sum of apparent molar concentration of reduced metalloporphyrin and reduced base metalloporphyrins
S_b = sum of apparent molar concentrations of uncombined base and base metalloporphyrins
T = absolute temperature
T = transmittance

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PART II. EXPERIMENTAL

Investigations by Barron (1), Clark, Taylor, Davies, and Vestling (2), Taylor (3), and Davies (4) have outlined several features of the coordination of metalloporphyrins with nitrogenous bases. There will now be reported potentiometric measurements with systems containing iron coproporphyrin, the properties of which have proved to be revealing, and also a few measurements with iron etioporphyrin, sufficient to check a point of some significance.

To establish a basis of comparison with the systems previously discussed the coordinating substances used were pyridine, nicotine, and cyanide. For the investigation reported here coproporphyrin I (1,3,5,7-tetramethyl-2,4,6,8-tetrapropionic acid porphine) and etioporphyrin I (1,3,5,7-tetramethyl-2,4,6,8-tetraethyl porphine) were synthesized. It is now possible to compare a series of systems in which the iron compounds of five different porphyrin structures are found. These are protoporphyrin IX, hematoporphyrin IX, mesoporphyrin IX, etioporphyrin I, and coproporphyrin I.

In addition, a preliminary study was made of the reversibility of the reaction

$$\text{Porphyrin} + \text{reduced metal ion}^{++} \rightleftharpoons \text{reduced metalloporphyrin} + 2H^+$$

in glacial acetic acid solution.

Preparations

Coproporphyrin I—The methods used were essentially those of Hans Fischer and coworkers (5). The procedure of Corwin and Quattlebaum (6) was used in the preparation of the starting material, 2,4-dimethyl-3,5-dicarbethoxypyrrole. Several modifications to be described were introduced in later steps which offered difficulty.

2,4-Dimethyl-3-carboxy-5-carbethoxypyrrole was obtained in 60 to 80 per cent yield from the 3-carbethoxy compound by treatment with concentrated sulfuric acid at 30°. Decarboxylation in glycerol (7) yielded 60 to 70 per cent of the next desired product, *2,4-dimethyl-5-carbethoxypyrrole* (m.p. 124°, uncorrected).

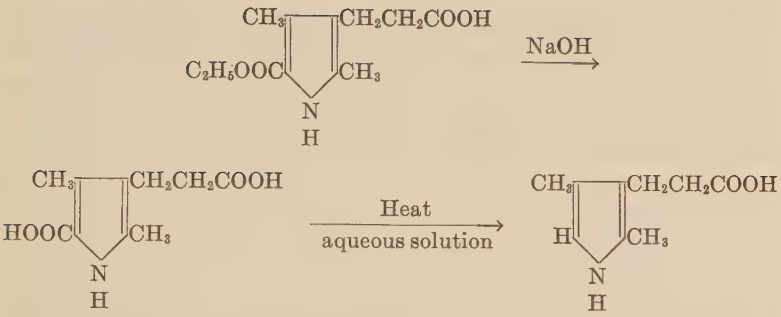
The modified Gattermann aldehyde reaction as described by Corwin and Andrews (8) was used to prepare 2,4-dimethyl-3-formyl-5-carbethoxypyrrole. The recrystallized pyrryl aldehyde melted sharply at 144–145° (uncorrected), and the yields varied from 60 to 77 per cent.

2,4-Dimethyl-3-formyl-5-carbethoxypyrrole was condensed with malonic acid in the presence of freshly distilled aniline (9) to give 60 to 80 per cent of *2,4-dimethyl-3-acrylic acid-5-carbethoxypyrrole* (m.p. 236–239°, with decomposition).

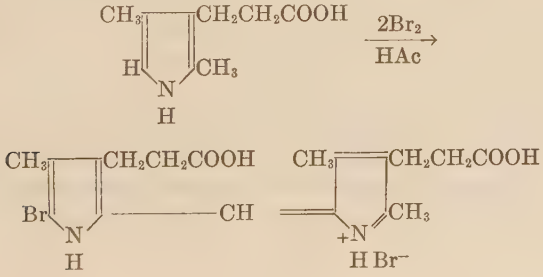
Catalytic hydrogenation with palladium chloride on charcoal (2 atmospheres of hydrogen) was found to be the most satisfactory method of reducing the acrylic acid side chain to the propionic acid side chain. The reduction went to completion (90 per cent yields) in alcohol solution, giving pure white *2,4-dimethyl-3-propionic acid-5-carbethoxypyrrole* (m.p. 158–159°, uncorrected) after recrystallization in alcohol. The pyrryl propionic acid is much more soluble in alcohol than the pyrryl acrylic acid; 200 ml. of alcohol and 17 gm. of the pyrryl acrylic acid were used in one preparation.

2,4-Dimethyl-3-propionic acid pyrrole (cryptopyrrolecarboxylic acid) was prepared as outlined below (10). These steps and the ones to follow offered great difficulty and the yields were very low.

A typical saponification and decarboxylation were carried out as follows: 3.3 gm. of purified carbethoxycryptopyrrolecarboxylic acid were placed in a 50 ml. Erlenmeyer flask equipped with a reflux condenser, 2.5 gm. of sodium hydroxide were added, and 20 ml. of distilled water. The mixture was heated on the steam bath for 3 hours, at the end of which time a slight yellow color was



noticeable. The cooled solution of the disodium salt was filtered into a small beaker, and further cooled by the addition of solid carbon dioxide. Then, with rapid stirring, concentrated hydrochloric acid was added drop by drop until the system was just acid to Congo red. Since both the free acid and its decarboxylation product were extremely unstable in air, solid carbon dioxide was kept present in the system and a nitrogen cone was used to exclude air as completely as practicable. The free carboxycryptopyrrolecarboxylic acid was obtained in good yield as a light yellow, crystalline product. On exposure to air it turned deep red in a few hours. The crude acid was transferred rapidly under nitrogen to a small beaker, water (10 ml. per 2 gm. of pyrrole acid) was added, and the system heated to boiling under nitrogen. Decarboxylation took place smoothly and the solution became red in color. When evolution of carbon dioxide had ceased, the system was rapidly cooled, filtered, and the product dried *in vacuo* over solid potassium hydroxide for several hours. The cryptopyrrolecarboxylic acid crystallized in beautiful light yellow needles of sharp melting point, 145–147° (uncorrected); yields varied from 40 to 66.7 per cent. Several hours exposure to air resulted in the formation of a deep red, water-soluble oil.



3′,4,5′-Trimethyl-3,4′-dipropionic acid-5-bromodipyrryl methene hydrobromide was prepared as described by Fischer and Fröwis (11), and fine red macroscopic crystals were obtained in 20 to 38 per cent yield (decomposition point, 220°, uncorrected). This compound was not analyzed, but was subjected to the final step in the synthesis.

Coproporphyrin I (1,3,5,7-tetramethyl-2,4,6,8-tetrapropionic acid porphine) (12) was obtained in poor yield by melting the methene hydrobromide in 3 times its weight of anhydrous succinic acid for 1 hour at 190°. Crude coproporphyrin I was obtained from the melt by grinding with dilute sodium hydroxide and filtering. The residue was extracted with strong hydrochloric acid until the extracts showed no more porphyrin spectrum. The hydrochloric acid and sodium hydroxide solutions were combined, sodium acetate was added nearly to saturation, and the pH adjusted to 4.6, with brom-cresol green as an indicator. On standing, a dark amorphous precipitate was obtained, which was centrifuged off, redissolved in dilute alkali, filtered, and reprecipitated several times. It was admittedly an impure product, but numerous attempts to crystallize the small amount at hand failed. From 0.8 gm. of methene hydrobromide about 0.25 gm. of total porphyrin was obtained. The bulk of the crude coproporphyrin I was made into the iron complex as described below, but a small amount was made into the tetramethyl ester for identification (9).

Coproporphyrin I tetramethyl ester was obtained in thick red-brown needles with a melting point of 250–253° (uncorrected, with decomposition). With the aid of a Zeiss hand spectroscope with calibrated wave-length scale and with the spectrophotometer the following absorption maxima were recorded:

Band I 492 to 508 mμ, Band II 524 to 538 mμ, Band III 560 to 570 mμ, Band IV 610 to 616 mμ. The order of intensity was Band I, II, III, IV.

Analysis of the recrystallized ester gave

$C_{40}H_{46}O_8N_4$. Calculated, C 67.60, H 6.47; found, C 67.04, H 6.95

The sample (10.341 mg.) left 1.33 per cent ash.

Etioporphyrin I (*1,3,5,7-Tetramethyl-2,4,6,8-Tetraethyl Porphine*) –The synthesis was carried out in this university by Walter Schlesinger for the most part according to the methods of Fischer

and Orth (5). The absorption maxima of the purified crystalline material in chloroform were as follows:

Band I 484 to 508 mμ, Band II 524 to 536 mμ, Band III 560 to 570 mμ, Band IV 610 to 618 mμ. The order of intensity was Band I, II, III, IV.

Analysis of the recrystallized material gave

$C_{32}H_{38}N_4$.	Calculated.	C 80.33, H 7.94, N 11.71
	Found.	" 80.99, " 8.17, " 11.83
		" 79.83, " 7.93

Preparation of Iron Copro- and Iron Etioporphyrins—Preparative methods for the formation of metalloporphyrins from porphyrins and metal ions and for the splitting of metalloporphyrins for the purpose of recovering their porphyrin constituents are suggestive of the reversibility of the reaction

$$\text{Porphyrin} + \text{metal ion}^{++} \rightleftharpoons \text{reduced metalloporphyrin} + 2H^+$$

Fischer, Treibs, and Zeile (13) and Treibs (14) have investigated this reaction and have presented evidence for this postulate. Oxidation of the reduced metalloporphyrin and the precipitation of the chloride (of hemin, for example) favor a shift "to the right" with increased production of metalloporphyrin. When the metal ion enters, it replaces 2 protons in the tetrapyrryl ring (15). It seems probable that a competition between metal ions and protons might turn in favor of the protons in solutions of high proton activity so that this reaction would appear to go from "right to left."

Since very limited supplies of coproporphyrin I and etioporphyrin I were available, it was desired to have some surety in the rationale of the method for preparing the iron compounds. Accordingly, the following experiment was conducted with the more abundant mesoporphyrin IX.

Glacial acetic acid buffer solutions had been prepared according to the method of Conant and Chow (16). However, since most of their buffer systems contain nitrogenous materials which are prone to coordinate with metalloporphyrins, inorganic buffers were used. These included 1 M sodium acetate, $(pH)^{HAc} = 3.70$; 0.5 M sodium acetate, $(pH)^{HAc} = 3.60$; 0.1 M sodium acetate, $(pH)^{HAc} = 3.34$; 0.1 M magnesium acetate, $(pH)^{HAc} = 3.01$; 0.2 M barium acetate, $(pH)^{HAc} = 2.73$; saturated zinc acetate,

$(pH)^{HAc} = 2.13$. These were measured according to the method of Conant and Chow.

The absorption bands of the species concerned, as observed with a Zeiss hand spectroscope, are as follows:

Mesoporphyrin IX in Buffered Glacial Acetic Acid—Band I 525 mμ, Band II 545 to 560 mμ, Band III 570 mμ, Band IV 590 to 605 mμ (diffuse). The order of intensity was Band II, IV, III, I.

Ferrimesoporphyrin IX in Buffered Glacial Acetic Acid—Band I 530 to 542 mμ (diffuse), Band II 630 to 640 mμ (sharp).

Ferromesoporphyrin IX in Buffered Glacial Acetic Acid—Band I 545 to 560 mμ, Band II 555 to 565 mμ (the intensities and definition about equal).

None of these absorption bands shifted with change of $(pH)^{HAc}$.

No splitting of ferric mesoporphyrin was observed when concentrated hydrochloric acid (5 ml.) was added to 10 ml. of a saturated solution, confirming the well known fact that an oxidized metalloporphyrin is resistant to splitting.

In a solution of $(pH)^{HAc}$ 3.7 reduction (hydrogen and colloidal palladium) and reoxidation (air) of the complex were performed several times without splitting. By the sixth reduction distinct traces of mesoporphyrin were detected. Since completely reversible oxidation-reduction was not observed in this, the most "alkaline" buffer, it was impossible to investigate the system potentiometrically.

It was not found practicable, with the limited method used, to decide upon a value of $(pH)^{HAc}$ centering the region on one side of which splitting is definitely favored and on the other side of which the coordination is definitely favored. Nevertheless, it was found that the higher the acidity of the solution the more the splitting was favored.

Reduction of ferrimesoporphyrin in unbuffered glacial acetic acid led in a few moments to mesoporphyrin and to the inability to reform the ferrimesoporphyrin.

These suggestive results indicate a distinct advantage in giving the glacial acetic acid solution a high value of $(pH)^{HAc}$ by the addition of the strong base, sodium acetate. Accordingly this was done in the preparation of iron coproporphyrin and iron etioporphyrin.

The procedure adopted is illustrated by the following preparation of iron etioporphyrin I chloride: 200 mg. of recrystallized

etioporphyrin I were placed in 175 ml. of 1 M sodium acetate in glacial acetic acid, about 3 gm. of sodium chloride were added, and the mixture warmed to aid solution. The system was then transferred to a special apparatus which consisted essentially of two small Erlenmeyer flasks equipped with reflux condensers and inlet and outlet tubes in such a way that tank nitrogen could be passed through both flasks. The entire operation to be described was carried out under nitrogen, and the two flasks were so arranged that the contents of one could be poured into the other through its reflux condenser. Ferrous acetate was prepared by refluxing glacial acetic acid over iron filings for a few moments under nitrogen in one flask; then about 50 ml. of the cooled, solution of ferrous acetate were added through a glass wool plug to the gently refluxing porphyrin solution. Immediately the color of the system changed from deep red to brown; after 10 minutes refluxing the system was opened to the air and let stand overnight. On standing, beautiful, macroscopic, octagonal platelets of metallic luster separated and were filtered off and washed repeatedly with 50 per cent acetic acid and then with water. If excess ferrous acetate had been added, no unchanged porphyrin was detectable. The yields in three such preparations were 88.1, 93.1, and 95.7 per cent of the theoretical.

In the case of iron coproporphyrin I complexes, solubility relationships modified the procedure. After the mixture was refluxed and had stood overnight, sufficient concentrated hydrochloric acid was added to react with the sodium acetate present. The system was then evaporated to dryness *in vacuo* and thoroughly extracted with 2 per cent hydrochloric acid, yielding a yellow filtrate of ferric chloride which exhibited no porphyrin or iron complex spectrum. The residue was a dark powdery material which was dried over phosphorus pentoxide. It was obviously impure as shown by the amounts of standardized, reduced phthiocol solution required in the titrations; it was estimated that the preparation contained about 70 per cent of iron coproporphyrin I complex. A colored impurity rendered spectrophotometric work impossible, but mathematical analysis of the titration data by the method of Reed and Berkson (17, 18) clearly demonstrated that the *main* product was iron coproporphyrin I chloride.

With this material reproducible potentiometric results were ob-

tained. To establish the identity of the coporporphyrin I, the tetramethyl ester was prepared and characterized as already stated. The reduced pyridine iron coproporphyrin I system in alkaline buffer solution showed the characteristic two-banded visible absorption with bands at 505 to 515 mμ and 540 to 550 mμ.

Description of Results

Descriptions of results will be abbreviated by referring to the propositions numbered in Paper I (2).

Potentiometric Measurements

The technique has been described in Papers I to III (2–4).

The observation of previous workers in this laboratory has been that the electrode potential is not steady in a solution of partially reduced metalloporphyrin if coordinating base be absent. In certain instances a sufficient reason is found in the progressive precipitation of one of the components (see Taylor (3)). On the other hand Davies (4) observed the same sort of drifts in systems the components of which were kept in solution by the addition of alcohol. Here the known difficulties with alcohol solutions might have contributed. With the water-soluble iron coproporphyrins we find drifts of potential of about the same rate and duration. The addition of a mediator did not improve matters.

Thus it is becoming evident that there is a distinct difference of behavior between metalloporphyrins and base metalloporphyrins. Apparently this is deep seated and will call for a special investigation.

Titrations—Tables I to III record typical titration data and are sufficiently representative to make unnecessary the recording of detail in all cases.

Symmetry of the titration curves indicated that p = 1 by Proposition I and rectification by Proposition XVI indicated that in each case $n = 1$. Hence the process is $OB_q + e \rightleftharpoons RB_r$.

The low solubility of the components of the iron etioporphyrin system necessitated the use of ethanol (75 per cent) and water mixtures. In such solutions progressive changes took place as was evident by the fading color. Rapid titrations yielded the data of Table IV which have every appearance of significance.

Relation of E_b to pH—The procedure with the "glass electrode"

has been described (2). Since the behavior of a glass membrane in buffer solutions having high concentrations of nitrogenous bases and alcohol is of especial importance to this investigation, various tests were made to insure the reliability of the measurements. It was found that the potentials with a glass membrane

TABLE I

Titration of Pyridine Ferricoproporphyrin I with Reduced Phthiocol at Constant pH and Constant Pyridine Concentration

Demonstration that $n = 1$. Pyridine = 1.05 M; total pigment $\fallingdotseq$ 4.23×10^{-5} M; phthiocol $\fallingdotseq$ 3.19×10^{-4} M; pH of borate buffer + pyridine = 9.99; $\mu = 0.1$; temperature = 30°; 100 per cent reduction at $y = 3.772$ ml.

y	$y - d^*$	Reduction	$0.0601 \times \log \frac{S_r}{S_o}$	E_h observed	E'_b calculated	Deviation from average
ml.	*ml.*	*per cent*		*volt*	*volt*	*volt*
0.80	0.28	8.59	−0.0617	−0.0013	(−0.0630)	−0.0058
1.01	0.49	15.03	−0.0452	−0.0132	(−0.0584)	−0.0012
1.22	0.70	21.47	−0.0338	−0.0230	−0.0568	+0.0004
1.42	0.90	27.61	−0.0251	−0.0313	−0.0564	+0.0008
1.64	1.12	34.36	−0.0169	−0.0404	−0.0573	−0.0001
1.88	1.36	41.73	−0.0087	−0.0487	−0.0574	−0.0002
2.12	1.60	49.09	−0.0009	−0.0567	−0.0576	−0.0004
2.36	1.84	56.45	0.0067	−0.0644	−0.0577	−0.0005
2.66	2.14	65.66	0.0169	−0.0746	−0.0577	−0.0005
3.02	2.50	76.71	0.0311	−0.0873	−0.0562	+0.0010
3.24	2.72	83.46	0.0422	−0.0961	(−0.0539)	+0.0033
3.50	2.98	91.43	0.0618	−0.1083	(−0.0465)	+0.0107
Average					−0.0572	

*Figures in the third decimal place of $y - d$ have not been recorded, since they are not physically significant, but, having appeared in the characteristics of the rectified curves, they were used automatically in the calculation of percentages of reduction.

were in orderly relation to the amounts of alkali added and were straight line functions of the alcohol concentration (to 80 per cent) in borate buffers with and without pyridine. In aqueous borate buffers the potentials were nearly straight line functions of the concentration of pyridine. It has been assumed *for purposes of comparison only* that the reproducible and orderly potentials

may be considered functions of the ratio of hydrogen ion activities on either side of the membrane as ordinarily formulated.

Fig. 1 shows that the pyridine iron etioporphyrin I system exhibits the relation $-dE_h/d$ pH = 0.0601 at 30°. This set of data is a decisive reinforcement of the argument that the carboxyl groups of the common metalloporphyrins have nothing to do with the "pH effect" within the range of pH shown. Etioporphyrin

TABLE II

Titration of Nicotine Ferricoproporphyrin I with Reduced Phthiocol at Constant pH and Constant Nicotine Concentration

Demonstration that $n = 1$. Nicotine = 0.22 M; total pigment $\fallingdotseq 5.6 \times 10^{-5}$ M; phthiocol $\fallingdotseq 5.31 \times 10^{-4}$ M; pH of phosphate buffer + nicotine = 11.25; $\mu = 0.1$; temperature = 30°; 100 per cent reduction at $y = 3.402$ ml.

y	$y - d$	Reduction	$0.0601 \times \log \frac{S_r}{S_o}$	E_h observed	E'_b calculated	Deviation from average
ml.	*ml.*	*per cent*		*volt*	*volt*	*volt*
0.21	0.45	12.31	−0.0512	−0.0880	(−0.1392)	−0.0017
0.46	0.70	19.18	−0.0375	−0.1000	−0.1375	0.0000
0.61	0.85	23.30	−0.0311	−0.1063	−0.1374	+0.0001
0.83	1.07	29.34	−0.0229	−0.1141	−0.1370	+0.0005
1.10	1.34	36.76	−0.0141	−0.1225	−0.1366	+0.0009
1.40	1.64	45.00	−0.0052	−0.1322	−0.1374	+0.0001
1.70	1.94	53.25	0.0034	−0.1408	−0.1374	+0.0001
2.00	2.24	61.49	0.0122	−0.1498	−0.1376	−0.0001
2.30	2.54	69.73	0.0218	−0.1601	−0.1383	−0.0008
2.61	2.85	78.25	0.0335	−0.1717	−0.1382	−0.0007
2.80	3.04	83.46	0.0422	−0.1814	(−0.1392)	−0.0017
3.00	3.24	88.96	0.0545	−0.1940	(−0.1395)	−0.0020
3.20	3.44	94.45	0.0740	−0.2130	(−0.1390)	−0.0015
Average					−0.1375	

See foot-note to Table I.

has no such groups. By inference a difference of 1 hydrogen or hydroxyl ion must be assigned to the coordination center of the reduced and oxidized forms of the metalloporphyrin. It is an arbitrary but reasonable assumption to ascribe a hydroxyl group to the coordination center of the oxidant. The symbol **OOH** will represent this assumption.

Fig. 1 presents the assembly of values of E_b obtained by in-

dividual titrations in the other aqueous solutions and shows the relation of potential to pH. The lack of strict conformity to the calculated curves may be attributed partly to experimental errors in the several measurements needed to place each experimental point, partly to the instabilities that are revealed by the sensitive potentiometric method and that involve some personal judgment of what an equilibrium potential is (potential drifts *less than*

TABLE III

Titration of Cyanide Ferricoproporphyrin I with Reduced Phthiocol at Constant pH and Constant Cyanide Concentration

Demonstration that $n = 1$. Cyanide = 0.019 M; total pigment $\fallingdotseq$ 5.6×10^{-5} M; phthiocol $\fallingdotseq$ 4.25×10^{-4} M; pH of borate buffer + sodium cyanide = 10.20; $\mu = 0.1$; temperature = 30°; 100 per cent reduction at $y = 3.075$ ml.; $d = 0$.

y	Reduction	$0.0601 \times \log \frac{S_r}{S_o}$	E_h observed	E'_b calculated	Deviation from average
ml.	*per cent*		*volt*	*volt*	*volt*
0.23	7.47	−0.0657	−0.1890	(−0.2547)	−0.0072
0.41	13.33	−0.0488	−0.2008	(−0.2496)	−0.0021
0.63	20.48	−0.0354	−0.2130	−0.2484	−0.0009
0.91	29.59	−0.0226	−0.2252	−0.2478	−0.0003
1.15	37.39	−0.0134	−0.2342	−0.2476	−0.0001
1.43	46.50	−0.0036	−0.2437	−0.2473	+0.0002
1.72	55.93	0.0062	−0.2535	−0.2473	+0.0002
2.05	66.66	0.0181	−0.2654	−0.2473	+0.0002
2.34	76.09	0.0302	−0.2773	−0.2471	+0.0004
2.63	85.52	0.0463	−0.2918	(−0.2455)	+0.0020
2.91	94.63	0.0749	−0.3092	(−0.2343)	+0.0132
Average				−0.2475	

0.0001 volt per minute), and partly to inadequacy of corrections in converting the data to a common basis. Such conversion involves not alone changing activity coefficients but also certain effects of larger order that will appear in the formulations given under "Discussion." Nevertheless, the principal feature seems to be adequately demonstrated. It is that systems containing nicotine and pyridine not only display the relation $-\Delta E_h/\Delta$ pH = 0.0601 in the more alkaline solutions, as Barron (1) and Davies

(4) found for similar systems containing other iron porphyrins, but they also display the transition to the "0 slope" which is a new feature.

By Propositions X and XI we may conclude that a species of oxidant associates with hydroxyl ion in the more alkaline solutions and not in the more acid solutions. It does not follow that the

TABLE IV

Titration of Pyridine Ferrietioporphyrin I with Reduced Phthiocol in 75 Per Cent Ethyl Alcohol-Borate Buffer Mixture at Constant pH and at Constant Pyridine Concentration

Demonstration that $n = 1$. Pyridine = 1.09 M; total pigment $\fallingdotseq 4.76 \times 10^{-5}$ M; phthiocol $\fallingdotseq 1.59 \times 10^{-4}$ M; pH of borate buffer + ethyl alcohol + pyridine = 12.29; temperature = 30°; 100 per cent reduction at $y = 6.483$ ml.

y	$y - d$	Reduction	$0.0601 \times \log \frac{S_r}{S_o}$	E_h observed	E'_b calculated	Deviation from average
ml.	*ml.*	*per cent*		*volt*	*volt*	*volt*
0.22	0.41	6.14	−0.0712	−0.1150	(−0.1862)	+0.0014
0.43	0.62	9.29	−0.0594	−0.1270	(−0.1864)	+0.0012
0.74	0.93	13.93	−0.0475	−0.1400	−0.1875	+0.0001
1.02	1.21	18.13	−0.0393	−0.1480	−0.1873	+0.0003
1.25	1.44	21.57	−0.0337	−0.1546	−0.1883	−0.0007
1.64	1.83	27.42	−0.0254	−0.1624	−0.1878	−0.0002
2.00	2.19	32.81	−0.0187	−0.1693	−0.1880	−0.0004
2.58	2.77	41.51	−0.0089	−0.1783	−0.1872	+0.0004
3.33	3.52	52.74	0.0028	−0.1903	−0.1875	+0.0001
3.85	4.04	60.54	0.0113	−0.1987	−0.1874	+0.0002
4.79	4.98	74.62	0.0281	−0.2159	−0.1878	−0.0002
5.28	5.47	81.97	0.0395	−0.2284	(−0.1889)	−0.0013
5.76	5.95	89.16	0.0550	−0.2424	−0.1874	+0.0002
Average					−0.1876	

See foot-note to Table I.

apparent pK_a values are necessarily those of the ionization alone. This will be discussed later.

The cyanide system is invariant with pH like the similar systems in which cyanide is the coordinating substance.

Association Curves—Association curves for the iron coproporphyrin system and pyridine are typified by the case shown in Fig. 2. By Proposition III, $K_O > K_R$. The change of potential

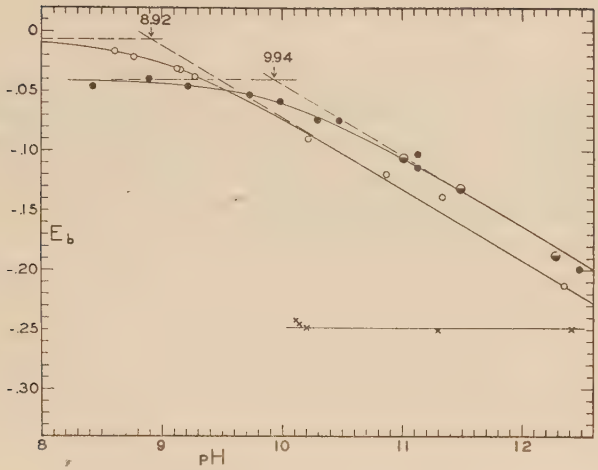


FIG. 1. Relation of E_b' to pH as determined by individual titrations. × represents cyanide iron coproporphyrin; $S_o = S_r \fallingdotseq 2.5 \times 10^{-5}$ M; cyanide $\fallingdotseq 0.02$ M; $t = 30°$; $\mu \fallingdotseq 0.1$; reducing agent, reduced phthiocol. ◒ represents pyridine iron etioporphyrin in water-ethanol (75 per cent), borate, and phosphate buffer solutions; $S_o = S_r \fallingdotseq 2.35 \times 10^{-5}$ M; pyridine $\fallingdotseq 1$ M; $t = 30°$; reducing agent, reduced phthiocol. ○ represents nicotine iron coproporphyrin; $S_o = S_r \fallingdotseq 2.5 \times 10^{-5}$ M; nicotine $\fallingdotseq 0.2$ M; $t = 30°$; $\mu \fallingdotseq 0.1$; reducing agent, reduced phthiocol. ● represents pyridine iron coproporphyrin; $S_o = S_r \fallingdotseq 2.5 \times 10^{-5}$ M (except at point pH = 11.16 where $S = 1 \times 10^{-4}$ M); pyridine $\fallingdotseq 1$ M; $\mu \fallingdotseq 0.1$; $t = 30°$; reducing agent, reduced phthiocol.

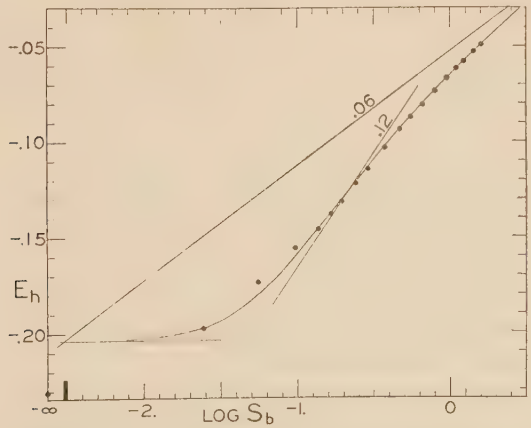


FIG. 2. Pyridine iron coproporphyrin. Relation of E_h to log S_b. The data are corrected to pH 10.02 (phosphate buffer) and 50 per cent reduction. $S = 5 \times 10^{-5}$ M; $t = 30°$. The empirical curve is drawn with $q = 1$, $r = 2$, $K_O = 0.6$, $K_R = 1.8 \times 10^{-3}$, $E'_0 = -0.2039$ volt (initial point neglected).

is so great as to signify that the ratio of K_O to K_R is large enough for the application of Proposition VI and the maximum slope then indicates that $r = 2$. Application of Propositions VII and VIII show that $q > 0$. The data are not extensive enough to make the approximate methods of analysis applicable with any certainty. Therefore empirical curve fitting was resorted to. The type curve

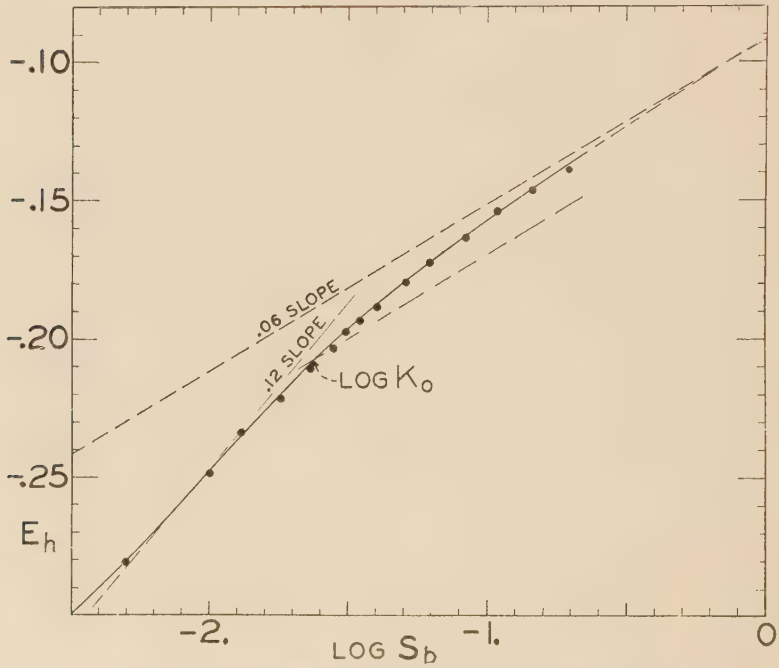


FIG. 3. Nicotine iron coproporphyrin. Relation of E_h to log S_b. $S \fallingdotseq 5 \times 10^{-5}$ M. The data are corrected to 50 per cent reduction and constant pH. pH = 11.14 (phosphate buffer); $\mu \fallingdotseq 0.1$. The curve is drawn with $q = 1$, $r = 2$, $K_O = 2.25 \times 10^{-2}$, $K_R = 3 \times 10^{-6}$, $E'_0 = -0.3245$ volt.

drawn with the constants indicated in the legend of Fig. 2 are to aid the eye in alignment of the experimental data, no reliance being placed on the estimate of K_O and K_R. K_R may be less than the value used for the empirical curve if the initial point be significant. The curve has been drawn with the assumption that $q = 1$, since there is no evidence that $q = 2$; but empirical curve fitting will succeed as well with the assumption that there is a stepwise associ-

ation and that the data extend only so far as to indicate the first step.

Empirical curve fitting had to be resorted to in dealing with the data of Fig. 3. Here it is much more certain that $r = 2$.

The case of cyanide association presented experimental difficulties. In several experiments the consequences appear in the poor alignment of the points. An attempt was made to stabilize the potentials by introducing indigotetrasulfonate as mediator. No distinct improvement followed. Fig. 4 represents one of the better series of measurements. Of particular interest is the decline of potential with increase in the concentration of cyanide

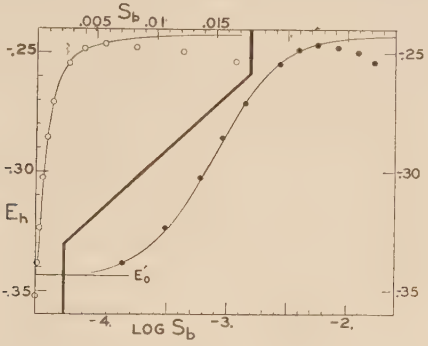


FIG. 4. Cyanide iron coproporphyrin. $S = 5 \times 10^{-5}$ M; $t = 30°$; per cent reduction $\fallingdotseq 50$. ○ represents the relation of E_h to S_b, ● relation of E_h to log S_b. The theoretical curves are drawn with $q = r = 2$, $K_O = 4 \times 10^{-6}$, $K_R = 8 \times 10^{-8}$, $E'_0 = -0.344$ volt (initial point neglected).

beyond about 0.005 M. Whether this be due to experimental error or to further addition of cyanide to the oxidant is uncertain. The over-all potential change is small, indicating a comparatively small ratio K_O/K_R. For this reason and also because of the uncertainties of points, graphical analysis is found to be unsatisfactory. Nevertheless, the theoretical curve given in Fig. 4 suggests that the main course of association follows that for the case of $q = r = 2$.

Comparison of Figs. 2, 3, and 4 shows that the value of K_R decreases in the order pyridine, nicotine, cyanide.

Spectrophotometric Measurements —The visible absorption bands of the oxidized metalloporphyrins change so little with addition

of pyridine or nicotine that the available spectrophotometer was inadequate for photometric measurements of the precision required for estimation of constants.

DISCUSSION

The data here presented confirm in principle several of the relations discussed by Taylor (3) and by Davies (4) and in particular they support Davies' theory of the decisive effect of competition between the nitrogenous bases and hydroxyl ion for bonds of the coordination shell.

We now meet a new feature that needs careful consideration. What is the significance of the distinctive bends of the E_b-pH curves of Fig. 1?

Since Davies' systems provided no evidence to the contrary, he postulated but one acid dissociation constant. Is it possible to account for the distinctive values of the apparent pK_a by use of his Equations 3 and 4 (4)? In the end a decision must rest upon the establishment of consistency among the numerical values of constants that are not yet available; but to provide a basis of discussion we may take a broad view by postulating the several species found in the following equations of equilibrium.

$$\frac{[R][B]^2}{[B_2R]} = K_R \quad (1) \qquad \frac{[BO][B]}{[B_2O]} = K_{O_1} \quad (2) \qquad \frac{[O][B]}{[BO]} = K_{O_2} \quad (3)$$

$$\frac{[O\overline{OH}][H^+]}{[O]} = K_{h_1} \quad (4) \qquad \frac{[B_2O\overline{OH}][H^+]}{[B_2O]} = K_{h_2} \quad (5) \qquad \frac{[BOOH][H^+]}{[BO]} = K_{h_3} \quad (6)$$

With these equations, and with obvious definitions of S_o and S_r, one may derive

$$E_h = E_0 + 0.06 \log \frac{S_o}{S_r} + 0.06 \log \frac{K_{O_1}K_{O_2}}{K_R} +$$

$$0.06 \log \frac{[H^+](K_R + [B]^2)}{K_{O_1}K_{O_2}([H^+] + K_{h_1}) + K_{O_1}[B]([H^+] + K_{h_3}) + [B]^2([H^+] + K_{h_2})} \quad (7)$$

When $[H^+]$ is much greater than K_{h_1} and K_{h_2} and K_{h_3}, and for any constant value of [B], the slope will be $-dE_h/d\ \mathrm{pH} = 0$.

When $[H^+]$ is much less than K_{h_1} and K_{h_2} and K_{h_3}, and for any constant value of [B], the slope will be $-dE_h/d\ \mathrm{pH} = 0.06$.

For each of these sets of conditions there will be a family of

straight lines. Those of any pair, representing a case of constant [B], will intersect where

$$[H^+] = \frac{K_{O_1}K_{O_2}K_{h_1} + K_{O_1}K_{h_3}[B] + K_{h_2}[B]^2}{K_{O_1}K_{O_2} + K_{O_1}[B] + [B]^2} \quad (8)$$

If [B] = 0, Equation 8 reduces to $[H^+] = K_{h_1}$; in other words the intersection of projections of the two branches of the E_h-pH curve is where pH = pK_{h_1}.

Should the terms in $[B]^2$ predominate in the sums of Equation 8, the intersection would be where pH = pK_{h_2}. Should the terms in [B] predominate in the sums of Equation 8, the intersection would be where pH = pK_{h_3}.

In the cases at hand the evidence is that the terms in $[B]^2$ have values of the second order and that [B] is somewhat greater than K_{O_2}. Therefore it would appear that the intersection is given approximately by $[H^+] = (K_{O_2}K_{h_1}/[B]) + K_{h_3}$ or in other words that it is considerably different from pH = pK_{h_1} and displaced a little from pH = pK_{h_3}.

Thus it would appear that the values of pK_a revealed in Fig. 1 are distinctive, largely, but not wholly, because they are determined by the nature of the associating base that enters together with hydroxyl ion to form the species $BO\overline{OH}$.

The case of the cyanide system can be reconsidered with Equation 7. Here the terms in $[B]^2$ predominate at high concentrations of cyanide. To obtain dE_h/d pH = 0, K_{h_2} must be much less than $[H^+]$. In other words the introduction of the negatively charged cyanide ions repels $\overline{OH}$ even at high concentrations of the latter.

Having thus mapped several of the gross features of these systems, we can now see several important relations that should be put to experimental test. It was the fate of this exploration that when the outline map had been completed the stock of coproporphyrin was exhausted.

SUMMARY

1. Some details of the synthesis of coproporphyrin I are mentioned.

2. A preliminary, spectroscopic study of the reaction

$$Fe^{++} + \text{porphyrin} \rightleftharpoons \text{ferroporphyrin} + 2H^+$$

in glacial acetic acid indicated that the "left to right" reaction is favored by low proton activity. Accordingly, the strong base sodium acetate was used in the preparation of ferricoproporphyrin I and ferrietioporphyrin I.

3. The oxidation-reduction potentials during titration of the pyridine, nicotine, and cyanide complexes of synthetic iron coproporphyrin I have been measured in alkaline, buffered, aqueous solutions and of pyridine iron etioporphyrin I have been measured in alkaline, buffered, ethanol (75 per cent) solutions. In every case the evidence indicates that all species are monomers and that 1 equivalent per mole is involved in the oxidation-reduction.

4. At constant, high concentration of pyridine the pyridine iron etioporphyrin system in aqueous ethanol exhibits the relation $-\Delta E_b/\Delta$ pH $= 0.06$. Since there are no carboxyl groups in this system, the result supports the assumption that a hydroxyl ion coordinates in the metal ion coordination shell.

5. At constant, high concentration of coordinating base the pyridine and nicotine iron coproporphyrin systems give $-\Delta E_b/\Delta$ pH $= 0.06$ at high pH. This value changes to 0 at low pH with apparent pK_a values of 9.9 for the pyridine system and 8.9 for the nicotine system.

6. At constant, high concentration of cyanide the cyanide iron coproporphyrin system gives $\Delta E_b/\Delta$ pH $= 0$.

7. The evidence is good that 2 molecules of cyanide, or pyridine or nicotine, associate with 1 of ferrocoproporphyrin. Orders of magnitude of the values of K_R are given. The values of K_R increase in the order cyanide, nicotine, pyridine.

8. It is certain that these bases coordinate with ferricoproporphyrin but the number (q) is uncertain in the cases of pyridine and nicotine although undoubtedly it is 2 in the case of cyanide.

9. To account for the distinctive, apparent dissociation constants of pyridine and nicotine ferricoproporphyrin it is shown that it is necessary to increase the number of components of Davies' model system.

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Vita

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